Original Article

Is a mix – A fix? "A microscopic analysis of depth of penetration of three combinations of irrigants"

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Abstract

Aims: The aim of the study is to evaluate the penetration depth of irrigants mixed with NaOCl into dentinal tubules at the apical third of the root canal using a confocal laser scanning microscope (CLSM).

Materials and Methods: Thirty-six single-rooted teeth were selected, decoronated, and straight-line access established. Then, the canal was enlarged with hand Protaper till size F3. The samples were divided randomly into three groups: Group 1 was irrigated with 3% NaOCl mixed with 1% phytic acid; Group 2 was irrigated with 3% NaOCl mixed with 18% etidronic acid; and Group 3 was irrigated with 3% NaOCl mixed with chitosan. The samples were subjected to CLSM evaluation. One-way analysis of variance with Tukey's *post hoc* was used for statistical analysis.

Results: Group 3 (NaOCl with chitosan) showed a greater depth of penetration in comparison to Groups 1 and 2.

Conclusion: Within limitations, it can be concluded that chitosan, in combination with NaOCl can be used as an alternative to the current irrigation protocol.

Keywords: Chitosan; etidronic acid; phytic acid

INTRODUCTION

Conventional endodontic therapy primarily aims at the elimination of microorganisms from the root canal space. The root canal system, being complex, presents areas that cannot be reached by mechanical instrumentation alone. Studies show that around 35%–53% of the canal space remains untouched after biomechanical preparation.[1,2] Here comes the role of irrigants in the effective debridement of complex areas of canal space such as fins, cul-de-sacs, and internal communications.[3] Among irrigants available today, NaOCl is considered the most effective and is most commonly used because of its tissue dissolving and antimicrobial properties. Ethylenediaminetetraacetic acid (EDTA) is also widely used because of its effective smear layer removal property. However, these two irrigants cannot be mixed as EDTA reduces the free active chlorine (FAC) ions of NaOCl, thereby hampering its antimicrobial properties. It is advocated to use these two irrigants sequentially, drying the canals in between their use. Studies, however, have shown that the alternative use of these two irrigants results in dentinal erosion.[4,5]

Etidronic acid (HEDP 1-hydroxyethylidene-1,1-diphosphonate) is a nontoxic, biocompatible soft chelator. Studies show that it can be mixed with NaOCl without the latter losing its

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desired chemical characteristics. This has also shown less demineralization kinematics than EDTA.[6,7]

Phytic acid, widely available in plant seeds and rice bran, has proven to be a potential chelating agent with multiple negative charges and has less demineralization effect compared to 17% EDTA.[8] Studies show that in combination with NaOCl, it causes better chelation.[9]

Chitosan is a nontoxic, natural polysaccharide with biocompatible, biodegradable, bioadhesion properties, usually extracted from the exoskeleton of crustaceans. Studies have demonstrated its significant antimicrobial and chelating properties with less alteration in radicular dentine.[10] The FAC content of NaOCl remains unaltered when it is mixed with chitosan.[11]

Literature shows that there are no other studies comparing the combination of the irrigants as mentioned above. Hence, the objective of this study is to compare the penetration depth of a combination of irrigants in dentinal tubules using a confocal laser scanning microscope (CLSM) at the apical third of the root.

MATERIALS AND METHODS

Sample preparation

A total of 36 freshly extracted, single-rooted teeth were taken, cleaned of debris, and stored in saline for use in the study. All teeth were decoronated with diamond discs at the level of the cementoenamel junction. Straight-line access to the apex was achieved and working length was determined, shaping and cleaning were carried out with a hand Protaper (Dentsply) up to F3 using saline between each instrument change.

Materials

1. NaOCl 3% (Prime)
2. 0.2% chitosan (Nanochemazone)
3. 1% phytic acid (TCI chemicals)
4. 18% etidronic acid (TCI chemicals).

Preparation of experimental solutions

- 1% phytic acid mixed with 3% NaOCl in 1:9 mixture
- 0.2% chitosan mixed with 3% NaOCl in 1:9 mixture
- 18% etidronic acid mixed with 3% NaOCl in a 1:9 mixture.

Then samples were divided randomly into three groups:

- Group A: NaOCl mixed with 1% phytic acid
- Group B: NaOCl mixed with 18% etidronic acid
- Group C: NaOCl mixed with 0.2% chitosan.

By using rhodamine B dye each irrigant was fluorescently labeled. Then, the experimental irrigating solution was used in the prepared canals and activated using ultrasonic tips. The canal was finally rinsed with saline. Each sample was mounted using self cure acrylic resin and a hard tissue microtome was used to get 1 mm sections at the apical third of the root. These were viewed under a CLSM.

Measurement of dentinal tubule penetration values using CLSM.

Sections viewed under CLSM AT 10 X AND 20 X MAGNIFICATION [Figure 2]. The maximum depth of penetration was measured from the root canal wall to the deepest point of irrigant penetration.

Statistical analysis

Data were analyzed using IBM SPSS version 20 software (IBM SPSS, IBM Corp., Armonk, NY, USA), and one-way analysis of variance with Tukey's *post hoc* was used for intergroup comparison at a significance level of $P < 0.05$.

RESULTS

Mean values of depth of penetration were shown in Table 1 and depicted in Figure 1.

Chitosan combination of NaOCl showed a statistically significant difference in comparison to groups 1 and 2, whereas Phytic acid combined with NaOCl showed a greater depth of penetration than group 2.

DISCUSSION

Studies in the literature show that the mixing of NaOCl and EDTA leads to a decrease in the pH of NaOCl in a time-dependent manner that affects free chlorine ions in the solution and increases chlorine gas and hypochlorous acid.[12,13] Studies also show that the alternative use of NaOCl and EDTA, drying canals in between their use resulted in dentinal erosion, affecting dentin flexural strength, and dentin microhardness.[14,15]

Etidronic acid, a weak chelator, can be mixed with NaOCl without compromising the antibiofilm or tissue dissolving properties of the latter. In addition, continuous chelation with the mixture results in less debris and smear layer accumulation.[16] This study also included phytic acid, as earlier studies stated that this has potential antibiofilm and antimicrobial properties.[17] When it is mixed with NaOCl it results in lesser chlorine depletion as compared to EDTA.[18] Chitosan is included in this study, because, apart from the aforementioned properties, studies have also shown that the FAC ions were not altered when combined with NaOCl.[9] Studies also showed that the application of EDTA resulted in a greater reduction in dentinal microhardness in comparison to phytic acid and chitosan.[19]

The objective of this study is to see if mixing these chelating agents with NaOCl can cause the irrigant to penetrate complex areas of the root canal, especially in the apical third, to achieve complete disinfection and subsequently facilitate a three-dimensional filling of those areas. The results of this study showed that NaOCl mixed with etidronic acid shows a lesser depth of penetration in comparison to the other two groups, whereas NaOCl mixed with chitosan shows a greater depth of penetration.

The combination of NaOCl with etidronic acid showed the least penetration depth. This may be due to weak HEDP - calcium complex formation (three times weaker than calcium chelating ability observed for EDTA). This is in tandem with studies conducted by Biel *et al*. and Wright *et al*.[20,21]

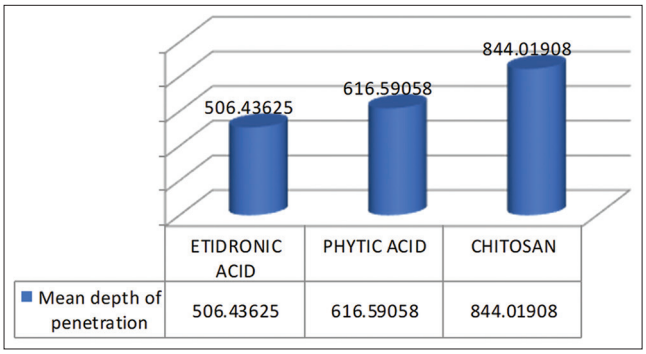

Figure 1: Mean depth of penetration in microns (μ)

NaOCl with phytic acid showed a greater penetration depth than with etidronic acid. This can be explained by the fact that it consists of multiple negative charges that increase its binding affinity to minerals, such as calcium and zinc, thereby providing a greater chelating effect. However, it showed a lesser depth of penetration in comparison to chitosan. This may be because the ability of phytic acid to bind with calcium depends on the number of phosphate substituents on the inositol ring. These results are in contrast to a study conducted by Puvvada *et al*., where the NaOCl and phytic acid combination showed significantly better chelating properties. This may be because this study was done using a calcium titration method.[9] In contrast, the present study used extracted teeth to simulate clinical conditions.

NaOCl with chitosan showed the highest depth of penetration. This may be due to the formation of a complex between chitosan and metal ions due to adsorption, ion exchange, and chelation. The mixture consists of a large number of free hydroxyl and amino groups that make it cationic in nature. This is responsible for the ionic interaction between dentinal calcium ions and the chelating agents. These results can be correlated with those of a study conducted by da Silva Mira *et al*. and a study conducted by Thota *et al*., which concluded that the depth of penetration of sealer at the apical third is greater when chitosan is used.[22,23]

To date, no other studies have been conducted to check the depth of penetration of admixed irrigants. Further

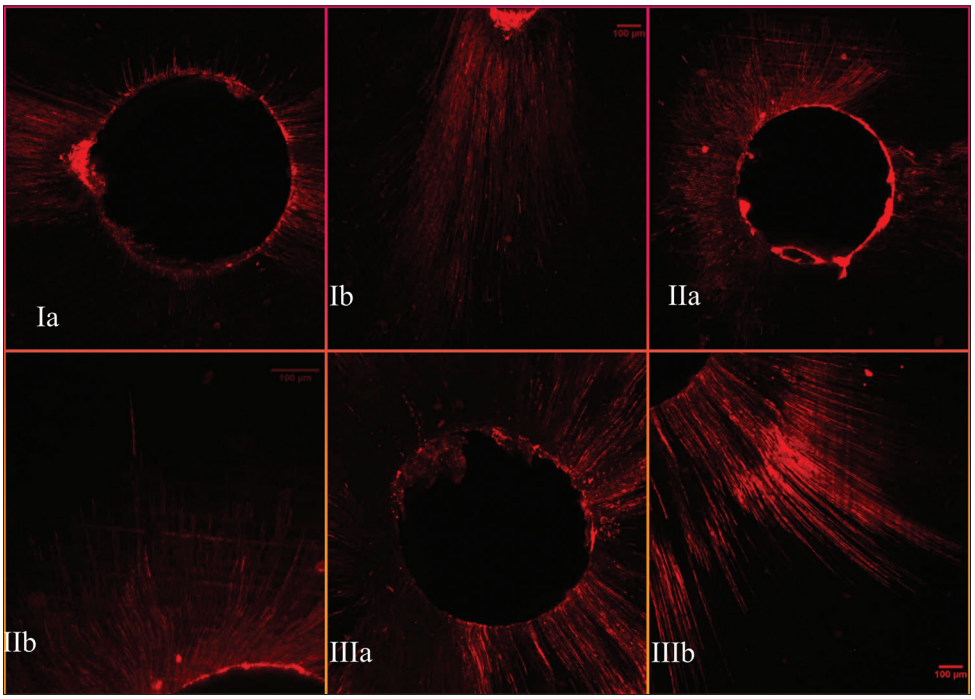

Figure 2: Ia - phytic acid group at 10× magnification. Ib - phytic acid group at 20× magnification. IIa - Etidronic acid group at 10× magnification. IIb - Etidronic acid group at 20× magnification. IIIa - Chitosan group at 10× magnification. IIIb – Chitosan group at 20x magnification

Table 1: Depth of irrigant penetration of respective groups

	n	Depth of penetration		*P*
		Mean	SD	
Etidronic acid combined with NaOCl	12	506.43625	125.517556	<0.001**
Phytic acid combined with NaOCl	12	616.59058	131.088805	
Chitosan combined with NaOCl	12	844.01908	168.843506	

**$P<0.001$ is considered statistically highly significant. SD: Standard deviation, NaOCl: Sodium hypochlorite

studies should be conducted to evaluate the antimicrobial and antibiofilm efficacy of these admixed irrigants.

Clinical relevance

To date, there is no single irrigant that possesses both tissue-dissolving properties and smear layer removal ability. This study on a combination of irrigants showed that chitosan combined with NaOCl is advantageous as it has a greater depth of penetration, which signifies that it can reach deeper areas of dentinal tubules, resulting in better disinfection. A single potent irrigating solution will simplify the irrigation procedure.

CONCLUSION

Within the limitations of this study, it can be concluded that chitosan combined with NaOCl shows a greater depth of penetration at the apical third. This is followed by phytic acid and the least penetration is seen with etidronic acid combined with NaOCl. Hence, we can conclude that chitosan mixed with NaOCl can be a promising single irrigating solution.

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Conflicts of interest

There are no conflicts of interest.

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